

Applicant : Stichting voor de Technische Wetenschappen  
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*C. J. de Vries*  
PCR amplifications and clonings are performed by standard procedures as described in Sambrook et al., Molecular Cloning; a laboratory manual, second edition.

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Please attach at the end of the application pages 1-3 of the Sequence Listing (attached hereto as Exhibit E).

REMARKS

By this Amendment, Applicant has amended the specification to refer to sequence identifiers, as required by the Sequence Rules, and to add the Sequence Listing. The amendments to the specification are supported by the application as originally filed. Accordingly, entry of the amendments to the specification is respectfully requested.

Compliance with Sequence Rules

The April 30, 2001 Notification to Comply With Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures (Exhibit D) indicated that the application did not comply with the Sequence Rules. In response thereto, Applicant attaches herewith Exhibit F, consisting of pages 1-3 of the Sequence Listing. Also enclosed is a computer-readable form containing the Sequence Listing (Exhibit G). Additionally, the specification has been amended to contain the correct sequence identifiers, as required by the Sequence Rules.

The undersigned attorney hereby certifies that the information recorded in computer-readable form is identical to the written Sequence Listing, is supported by the application as filed, and does not introduce new matter into the application as filed. In view of the above-noted amendments and these remarks, applicant respectfully submits that he has complied with the Sequence Rules. Accordingly, entry of the Sequence Listing is respectfully requested.

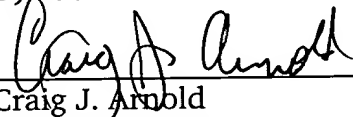
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No fee is deemed necessary in connection with the filing of this Amendment. If any fee is required to preserve the pendency of the application, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 01-1785.

Respectfully submitted,

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June 29, 2001

By:   
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SCHEDULE AREDLINED VERSIONIn the Specification:

Please replace the paragraph at page 7, line 11 as follows:

A vector coding for EBNA-1 (a nuclear antigen) is the hygromycin resistance gene comprising pREP4 vector (Invitrogen Corporation, Carlsbad, USA). The ~~De~~ EBNA-1 sequence is present to ensure that the vector does not (stably) integrate in the genome, but replicates episomally. The promoter ~~promoter~~ (Prsv) of this vector has been removed by digestion with the restriction enzyme Sall and replaced by a synthesized sequence having four binding sites for LexA from *E. coli*. This sequence is from 5' - 3' : GTCGACTGCTGTATATAAAACCAGTGGTTATATGTAC AGTACTTGTACTGTACATATAACCACTGGTTTTATATACAGCAAGCTTGGATCCGT CGAC (SEQ ID NO:1). The 5' side of this sequence comprises a Sall site, the 3' side a HindIII-BamHI-Sall site (all shown in bold type). Downstream from the LexA binding sites in the HindIII and BamHI sites, the human heat shock factor-inducible promoter ~~promoter~~ (0.29 kbp HindIII/NcoI fragment) and the luciferase reporter gene inclusive of SV40 polyadenylation signal (1.9 kbp NcoI/BamHI fragment) are cloned in a three-way ligation. The human heat shock factor-inducible promoter ~~promoter~~ (hsp70; accession numbers M59828 and M34267; nucleotides 52 to 244) can be obtained by means of PCR amplification on human genomic DNA (Cat. No. 6550-1; Clontech, Palo Alto, USA). As PCR primers, forward primer 5' - 3' : AAGCTTGGGAG TCGAAACTTCTGGAATATTCCCGAACTTTCAGCCGACGACTTATAAAACGCCA GGGGCAAGC (SEQ ID NO:2) may be considered; and as reverse primer 5' - 3' : CCATGGTTTAGCTTCCTTAGCTCCTGAAAATCTCGCCAAGCTCCCGGGGTCCGCGAG AAGAGCTCGGTCCTTCCGG (SEQ ID NO:3). The forward primer comprises a HindIII site, the reverse primer comprises a NcoI site (given in bold print). The luciferase reporter gene inclusive of SV40 polyadenylation signals were obtained through

NcoI/BamHI digestion of the pGL3 control vector (Cat. no E1741; Promega, Madison, USA). In the thus obtained vector, in the HindIII site between the LexA binding sites and the heat shock promoter ~~promotor~~, either a 2.1 kbp HindIII fragment of phage lambda is cloned (Pharmacia Biotech, Uppsala, Sweden), or a 1.7 kbp scs HindIII fragment. The 1.7 kbp scs DNA fragment is isolated from genomic *Drosophila* DNA (Cat. #6940-1, Clontech, Palo Alto, USA) with the aid of PCR primers (Forward primer 5' - 3' : GATCAAGCTTATGATCTGCGTATGATACCAAATTTCTG (SEQ ID NO:4); Reverse primer 5' - 3' : GACAAGCTTACATTGCTGGGCGAGCTGCGCCAATCG (SEQ ID NO:5)). At the ends of these primers HindIII restriction enzyme sites were located. The vector with the Lambda fragment (control) is indicated as reporter construct a, the vector with the scs fragment as reporter construct b. Restriction enzyme digestions, PCR amplifications and clonings are performed by standard procedures as described in Sambrook et al., Molecular Cloning; a laboratory manual, second edition.